Selective Targeting of Glucagon-Like Peptide-1 Signalling As a Novel Therapeutic Approach for Cardiovascular Disease in Diabetes

SELECTIVE TARGETING OF GLUCAGON-LIKE PEPTIDE-1 SIGNALLING AS A NOVEL THERAPEUTIC APPROACH FOR CARDIOVASCULAR DISEASE IN DIABETES

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SUMMARY

Glucagon-like peptide-1 (GLP-1) is an incretin hormone whose glucose-dependent insulinotropic actions have been harnessed as a novel therapy for glycaemic control in type 2 diabetes. Although it has been known for some time that the GLP-1 receptor is expressed in the cardiovascular system where it mediates important physiological actions, it is only recently that specific cardiovascular effects of GLP-1 in the setting of diabetes have been described. GLP-1 confers indirect benefits in cardiovascular disease (CVD) under both normal and hyperglycaemic conditions via reducing established risk factors, such as hypertension, dyslipidaemia and obesity, which are markedly increased in diabetes. Emerging evidence indicates that GLP-1 also exerts direct effects on specific aspects of diabetic CVD, such as endothelial dysfunction, inflammation, angiogenesis and adverse cardiac remodelling. However, the majority of studies have employed experimental models of diabetic CVD and information on the effects of GLP-1 in the clinical setting are limited although several large-scale trials are ongoing. It is clearly important to gain a detailed knowledge of the cardiovascular actions of GLP-1 in diabetes given the large number of patients currently receiving GLP-1 based therapies. This review will therefore discuss current understanding of the effects of GLP-1 on both cardiovascular risk factors in diabetes and direct actions on the heart and vasculature in this setting, and the evidence implicating specific targeting of GLP-1 as a novel therapy for CVD in diabetes.

Abbreviations: ANP (atrial natriuretic peptide); CAD (coronary artery disease); cAMP (cyclic adenosine monophosphate); CVD (cardiovascular disease); DPP-4 (dipeptidyl peptidase-4); eNOS (endothelial nitric oxide synthase); ERK (extracellular signal-regulated kinase); GLP-1 (glucagon-like peptide-1); GLP-1R (glucagon-like peptide-1 receptor); hs-CRP (high sensitivity C-reactive protein); HUVEC (human umbilical vein endothelial cell); ICAM-1 (intercellular adhesion molecule-1); MAPK (mitogen-activated protein kinase); MI (myocardial infarction); MMP (matrix metalloproteinase); NF-κβ (nuclear factor kappa-light-chain-enhancer of activated B); NO (nitric oxide); PAI-1 (plasminogen activator inhibitor-1); PI3K (phosphoinositide 3-kinase); PKA (protein kinase A); STZ (streptozotocin); T1DM (type 1 diabetes mellitus); T2DM (type 2 diabetes mellitus); TLR (toll-like receptor); TNF-α (tumour necrosis factor-α); UKPDS (United Kingdom Prospective Diabetes Study); VCAM-1 (vascular cell adhesion molecule-1).

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is increasing alarmingly with the 2013 figure of 382M estimated to rise to 592M by 2035 (International Diabetes Federation, 2014). A change in lifestyle coupled with an increase in obesity has led to a global epidemic, with diabetics typically carrying a 5-fold greater mortality risk as a result of cardiovascular disease (CVD) compared with non-diabetics (Stamler et al., 1993), with coronary artery disease (CAD) being the leading underlying cause (Bertoni et al., 2004). It is well established that hyperglycaemia plays a central role in development and progression of CVD associated with diabetes (Nathan, 1996). Indeed, two long-term clinical trials, the Diabetes Control and Complications Trial / Epidemiology of Diabetes Interventions and Complications study and the United Kingdom Prospective Diabetes Study (UKPDS), have demonstrated that intensive glucose-lowering strategies are effective in markedly reducing the incidence of microvascular (e.g. retinopathy, nephropathy) and macrovascular (e.g. CAD, stroke) complications in both type 1 diabetes mellitus (T1DM) and T2DM (Holman et al., 2008), although several similar large-scale trials have reported limited benefits (Action to Control Cardiovascular Risk in Diabetes Study Group, 2008; Duckworth et al., 2009; Ginsberg, 2011). Nonetheless, there remains a significant incidence of CVD even in optimally-treated diabetic patients so it is clear that more effective strategies are required. In this regard, the incretin peptide hormone, glucagon-like peptide-1 (GLP-1), has received considerable recent attention.

The incretin effect is responsible for augmenting insulin secretion following nutrient ingestion, and GLP-1 together with its sister hormone, gastrointestinal peptide, account for up to 60% of post-prandial insulin secretion, leading to rapid blood glucose reduction (Nauck et al., 1986; Drucker et al., 1987). Furthermore, they possess an inherent ability to reduce glucagon secretion (Kreymann et al., 1987), delay gastric emptying (Näslund et al., 1998a) and promote satiety (Flint et al., 1998). The metabolic actions of GLP-1 are mediated by

GLP-1 receptor (GLP-1R) activation and stimulation of cyclic adenosine monophosphate (cAMP) and several downstream kinases, including extracellular signal-regulated kinase (ERK) 1/2, phosphoinositide 3-kinase (PI3K), and protein kinase A (PKA). Under physiological conditions GLP-1 has a short half-life (~2 minutes) as it is rapidly degraded by its endogenous inhibitor, dipeptidyl peptidase-4 (DPP-4) (Deacon et al., 1995), resulting in cleavage of two amino acids from native GLP-1(7-36) to produce GLP-1(9-36), which acts as a weak GLP-1R antagonist lacking insulinotropic activity (Green et al., 2004). However, emerging evidence suggests that "metabolically-inactive" GLP-1(9-36) may itself be an important signalling molecule (Ban et al., 2010; Gardiner et al., 2010). More detailed information on GLP-1 biology and signalling is provided by recent review articles (Grieve et al., 2009; Donnelly, 2012; Pabreja et al., 2013).

The unique ability of GLP-1 to promote insulin secretion in a glucose-dependent manner has been harnessed for treatment of T2DM, with GLP-1R agonists resistant to DPP-4 (exenatide, Byetta®; liraglutide (Victoza®), and DPP-4 inhibitors (e.g. sitagliptin, vildagliptin) now widely used for effective glycaemic control. Interestingly, it is well recognised that GLP-1 exerts wide-ranging extra-pancreatic actions occurring independently of its established metabolic effects. Indeed, GLP-1 signalling is reported to play several important roles in the cardiovascular system in both health and disease (Grieve et al., 2009), although it appears that the GLP-1R may not be as widely expressed as previously thought. For example, recent work suggests that cardiac GLP-1R expression may be localised to atrial tissue, sino-atrial node and vasculature, with some species variation (Kim et al., 2013; Pyke et al., 2014; Richards et al., 2014), and that earlier reports of more ubiquitous expression may be questionable due to poor antibody selectivity and sensitivity (Panjwani et al., 2012). Nonetheless, it is clear that GLP-1 exerts important cardiovascular actions, although it is only recently that its effects in the setting of diabetes, a condition synonymous with micro/macrovascular complications, have

been explored. This is clearly important due to the large number of patients receiving GLP-1 based therapies, in which its cardiovascular actions are largely unknown. This review will therefore discuss current understanding of the effects of GLP-1 on both cardiovascular risk factors in diabetes and direct actions on the heart/vasculature in this setting, and the evidence implicating specific targeting of GLP-1 as a novel therapy for CVD in diabetes, with a primary focus on the role of GLP-1R agonists. More detailed discussion of the pleiotropic actions of DPP-4 inhibitors in this setting, is provided by recent review articles specifically focused on this important aspect of cardiovascular GLP-1 signalling (Scheen, 2013; Aroor et al., 2014).

INFLUENCE OF GLP-1 ON CARDIOVASCULAR RISK FACTORS IN DIABETES

Blood pressure and hypertension

Increased blood pressure is an established risk factor for CVD in both normoglycaemia and T2DM (Turner et al., 1998; Vasan et al., 2001). Notably, therapeutic reduction of blood pressure and circulating glucose has an additive effect in decreasing cardiovascular complications in T2DM patients, as highlighted by the UKPDS (Stratton et al., 2006). Indeed, in an experimental setting, chronic GLP-1 infusion inhibits development of hypertension in Dahl salt-sensitive rats, as well as reducing cardiac fibrosis and hypertrophy, effects which appear to occur via a natriuretic/diuretic mechanism independently of blood glucose (Yu et al., 2003), suggesting that GLP-1 may confer additional benefits which could be harnessed for the treatment of hypertension associated with T2DM. Consistent with an indirect blood pressure-lowering effect, it was recently reported that liraglutide-stimulated reduction of angiotensin II-induced hypertension in mice was blocked by the natriuretic peptide receptor antagonist, ananin, in a GLP-1R dependent manner, but unaltered by the nitric oxide (NO) synthase inhibitor, L-NMMA, and that liraglutide induced rapid increases in

atrial natriuretic peptide (ANP) secretion both *in vivo* and in isolated perfused hearts, suggesting that observed blood pressure reduction occurred at least partly via direct activation of cardiac ANP (Kim et al., 2013). Importantly, in the context of diabetes, the GLP-1 mimetic, exendin-4, inhibited development of both spontaneous and high salt-induced hypertension in obese db/db mice via beneficial actions on renal sodium handling (Hirata et al., 2009). Furthermore, it was recently reported that treatment of insulin-resistant Zucker rats with the DPP-4 inhibitor, linagliptin, for 8 weeks reduced blood pressure and improved diastolic function (Aroor et al., 2013).

Interestingly, although chronic administration of GLP-1 may prevent development of hypertension, it is widely reported that acute GLP-1 exposure is associated with increased blood pressure and heart rate, which predisposes to CVD. For example, acute infusion of GLP-1(7-36) increased systolic/diastolic blood pressure and heart rate in both normal and insulin-deficient streptozotocin (STZ)-induced T1DM rats (Barragan et al., 1994), with the same group reporting that exendin-4 induced increases in blood pressure and heart rate were reversed by the GLP-1R antagonist, exendin(9-39) (Barragan et al., 1996), suggesting that these effects occurred via an insulin-independent mechanism but involving GLP-1R activation. Although similar increases in blood pressure and heart rate after short-term GLP-1 administration have been reported in various experimental models (Grieve et al., 2009), the data from clinical studies are less clear. For example, GLP-1 infusion in a small number of T2DM patients with or without CAD for 105 minutes and 48 hours, respectively, had no effect on heart rate or systolic/diastolic blood pressure (Toft-Nielsen et al., 1999; Nyström et al., 2004), whereas 48-hour GLP-1 infusion in patients with ischaemic heart failure (Halbirk et al., 2010), and treatment of T2DM patients with an exendin-transferrin fusion protein or exenatide for 7 or 10 days, respectively (Kothare et al., 2008; Gustavson et al., 2011), resulted in elevated heart rate and diastolic blood pressure. However, the data from longer-term GLP-1

clinical trials are more consistent, with the majority reporting decreased blood pressure and minimal effects on heart rate. For example, the LEAD-4 (Liraglutide Effect and Action in Diabetes) study, investigating 26-week liraglutide treatment in combination with metformin in T2DM patients, reported a modest reduction in systolic blood pressure of 6mmHg compared to placebo (1.1mmHg) (Zinman et al., 2009). Similarly, the LEAD-2 study, assessing liraglutide combination therapy, reported a 2-3mmHg decrease in systolic blood pressure versus a small increase (0.4mmHg) in the glimepiride control group (Nauck et al., 2009). Furthermore, a 30-week trial comparing once-weekly versus twice-daily exenatide injection in drug-naïve T2DM patients observed a significant decrease in systolic/diastolic blood pressure compared to baseline (Drucker et al., 2008), whilst another 20-week trial in obese patients reported reduced systolic/diastolic blood pressure in response to liraglutide, which persisted for the 2 year follow-up period (Astrup et al., 2012). Interestingly, meta-analysis of 6 clinical trials comprising 2171 T2DM patients found that exenatide treatment for 6 months produced maximal systolic blood pressure reduction in individuals with abnormally high baseline levels, whereas no effects were observed in normotensive subjects (Okerson et al., 2010). It should be noted that blood pressure reduction is positively correlated with weight loss (Neter et al., 2003), so it is possible that the observed changes after chronic GLP-1 treatment may occur secondary to its metabolic effects. However, although beneficial effects of GLP-1 on body weight are associated with improved hypertension, it is clear that this cannot solely account for its vascular effects as several studies have reported blood pressure reduction prior to weight loss. For example, a combined meta-analysis of three 26-week liraglutide trials reported decreased blood pressure after only 2 weeks, whilst maximal weight loss did not occur until 8 weeks (Gallwitz et al., 2010). Indeed, heart rate, which is not linked to body weight, was increased by chronic administration of both liraglutide and exenatide in T2DM patients in parallel with reduced systolic blood pressure (Garber et al., 2009; Gill et

al., 2010). Interestingly, it was recently reported that GLP-1 secretory function increases with age and is negatively correlated with systolic blood pressure, suggesting that this may represent an adaptive response (Yoshihara et al., 2013).

Dyslipidaemia

The pathophysiology of diabetes is commonly considered largely in terms of associated hyperglycaemia. However, it is increasingly apparent that dyslipidaemia is equally important and represents a significant risk factor for CVD in diabetic patients (Reiner et al., 2011). It is likely that impaired insulin sensitivity contributes to dyslipidaemia in T2DM, which is associated with reduced GLP-1 secretion. Indeed, in addition to their established glycaemic actions, GLP-1R activation and DPP-4 inhibition are reported to improve lipid profiles in both experimental and clinical diabetes. For example, short-term infusion of GLP-1 in normoglycaemic Syrian golden hamsters decreased lipid absorption and triglyceride levels, an effect potentiated by oral glucose (Hein et al., 2013), suggesting that its incretin action may inhibit intestinal production of chylomicrons, which are strongly linked to atherosclerosis (Nakano et al., 2008). Similarly, circulating triglycerides and fat pad mass in rats with diet-induced obesity were reduced after 4-week treatment with liraglutide (Madsen et al., 2010), whilst 40-day administration of exendin-4 ameliorated systemic and cardiac insulin resistance and dyslipidaemia in both genetic KKAy and diet-induced T2DM mouse models (Monji et al., 2013). Furthermore, chronic GLP-1R activation with both the GLP-1 analogue, CNTO3649, and exendin-4 in apolipoprotein E3-Leiden transgenic mice, which develop severe hypercholesterolaemia after high-fat feeding, resulted in reduced VLDL-triglyceride and apolipoprotein B synthesis in parallel with decreased hepatic triglyceride, cholesterol and phospholipids and lipogenesis gene expression (Parlevliet et al., 2012). The long-acting DPP-

4 inhibitor, teneligliptin, is also reported to decrease circulating triglyceride and free fatty acid levels in insulin-resistant Zucker fatty rats after 2-week treatment (Fukuda-Tsuru et al., 2012).

Importantly, the majority of clinical studies have also demonstrated beneficial outcomes of GLP-1 administration on lipid metabolism in T2DM, such as reduced circulating triglycerides and LDL cholesterol (Flock et al., 2007; Drucker et al., 2008; Tremblay et al., 2011), although one study in which patients were treated with exenatide for 24 weeks reported a similar plasma lipid profile versus controls (Moretto et al., 2008). For example, decreased circulating levels of atherogenic triglyceride-rich lipoproteins were observed following 4-week vildagliptin monotherapy in drug naïve T2DM patients, characterised by specific reductions in total plasma and chylomicron triglycerides, together with apolipoprotein B-48 and cholesterol in the chylomicron sub-fraction (Matikainen et al., 2006). Furthermore, the LEAD-4 study found that 26-week liraglutide treatment in combination with metformin and rosiglitazone decreased circulating LDL cholesterol, triglycerides and free fatty acids in T2DM patients compared to placebo controls, although it is interesting to note that these changes were greater in response to low-dose treatment (Zinman et al., 2009). Indeed, similar results are reported for DPP-4 inhibitors, which produce much lower circulating levels of GLP-1. For example, twice-daily sitagliptin led to significant reduction of circulating triglycerides and free fatty acids in a large number of T2DM patients compared to placebo and glipizide control groups, despite similar decreases in fasting plasma glucose and HbA1C levels (Scott et al., 2007). Interestingly, a single injection of exenatide was shown to attenuate postprandial increases in triglycerides, apolipoprotein B-48 and CIII/remnant lipoprotein cholesterol for up to 8 hours in patients with impaired glucose tolerance and recent-onset T2DM (Schwartz et al., 2010), suggesting that such lipid profile benefits may not be explained solely by chronic changes in body weight, glucose levels and insulin resistance. Indeed, it was recently reported that exendin-4 completely reverses hepatic steatosis in mice

fed a high-fat diet via a GLP-1R dependent mechanism resulting in reduced numbers/size of circulating VLDL-triglyceride and VLDL-apolipoprotein B particles (Parlevliet et al., 2012), suggesting that GLP-1 may exert direct effects on dyslipidaemia in diabetes.

Obesity

Although it is well known that obesity significantly increases risk of T2DM (Willett et al., 1999), and both are independent risk factors for CVD (Hubert et al., 1983), many established diabetes therapies, including sulfonylureas and thiazolidinediones, may increase body weight. However, GLP-1 reduces body weight due to beneficial effects on glucagon secretion, gastric emptying and satiety (Kreymann et al., 1987; Flint et al., 1998; Näslund et al., 1998a), so it seems likely that impaired GLP-1 secretion observed in non-diabetic obese individuals (Holst et al., 1982; Näslund et al., 1998b), may at least partly account for their increased body weight. Indeed, weight loss improves the postprandial GLP-1 response in severely-obese patients (Verdich et al., 2001), suggesting that the two are interlinked. Furthermore, 20-week treatment with liraglutide was reported to cause significant weight loss in obese individuals and to reduce incidence of pre-diabetes (Astrup et al., 2009), confirming an apparent role for GLP-1 in weight control which may be harnessed for therapeutic benefit. This assertion is supported by the LEAD trials which have consistently reported body weight reduction in T2DM patients following liraglutide treatment (Moretto et al., 2008; Buse et al., 2009; Garber et al., 2009; Nauck et al., 2009, 2013; Zinman et al., 2009). For example, in the LEAD-2 trial, 26-week combination therapy of liraglutide with metformin in T2DM patients resulted in increased weight loss compared to metformin alone (Nauck et al., 2013). Importantly, weight loss associated with both liraglutide and exenatide treatment is reported to be linked with improved cardiovascular risk factors, such as HbA1c and blood pressure, and to persist for at least two years (Klonoff et al., 2007; Astrup et al., 2009, 2012),

highlighting important benefits of GLP-1 which may not be related to its insulinotropic actions. The long-term effects of GLP-1 on weight loss may be particularly important as conventional weight loss is typically poorly maintained in T2DM patients. Despite GLP-1R agonists promoting weight loss in both diabetic and non-diabetic obese subjects, DPP-4 inhibitors appear to be weight-neutral (Amori et al., 2007), suggesting that GLP-1R agonists may exert direct gastrointestinal effects in addition to improving insulin resistance (Rask et al., 2001), although this could simply be due to differences in circulating GLP-1 levels. However, postprandial GLP-1 levels are reported to be increased immediately after gastric bypass surgery, despite patients remaining obese, indicating that GLP-1 may regulate appetite and food intake directly (Morinigo et al., 2006). Indeed, in T2DM patients GLP-1 promotes satiety, thereby reducing energy consumption (Gutzwiller et al., 1999), whilst in healthy individuals intravenous administration of GLP-1(7-36) slows gastric emptying in a dose-dependent manner (Nauck et al., 1997).

GLP-1 AND VASCULAR DISEASE

Vascular function

Impaired endothelial and vascular function are established as key initiating factors underlying development of microvascular and macrovascular complications associated with diabetes. Indeed, it has been known for some time that native GLP-1(7-36) induces *ex vivo* dose-dependent vasodilatation in a number of isolated rodent vessels, including aorta (Golpon et al., 2001; Green et al., 2008), pulmonary artery (Richter et al., 1993; Golpon et al., 2001), femoral artery (Nyström et al., 2005), and mesenteric artery (Ban et al., 2008), although several different mechanisms have been proposed. For example, some studies indicate that the vasorelaxant actions of GLP-1 are dependent upon endothelium-derived NO (Golpon et al., 2001; Ban et al., 2008; Gaspari et al., 2011), whereas others have proposed endothelium-

independent mechanisms involving mediators such as K_{ATP} channels, cAMP and β_2-adrenoceptor activation (Nyström et al., 2005; Gardiner et al., 2008; Green et al., 2008). Interestingly, although several studies suggest that the vascular actions of GLP-1 are dependent upon the GLP-1R (Gaspari et al., 2011; Chai et al., 2012), it appears that they may also be mediated, at least partly, by its truncated metabolite, GLP-1(9-36), which induces dose-dependent relaxation in both isolated mouse mesenteric artery (Ban et al., 2008) and rat aorta (Green et al., 2008). It should be noted that although the synthetic GLP-1 mimetic, exendin-4, exerts similar actions in rat aorta (Golpon et al., 2001; Green et al., 2008), they are of reduced magnitude compared to GLP-1(7-36) and are absent in mouse mesenteric artery (Ban et al., 2008). Importantly, the vasorelaxant actions of GLP-1 are also reported *in vivo*. For example, systemic administration of GLP-1(7-36) by both bolus dose and short-term infusion in rats induced hindquarters vasodilation (Gardiner et al., 2010). Interestingly, however, GLP-1 promoted vasoconstriction in both mesenteric and renal arteries, whilst exendin-4 exerted similar vasoconstriction in mesenteric artery but induced vasodilatation in both hindquarters and renal artery (Gardiner et al., 2008), suggesting differential vascular effects. Indeed, a similar study demonstrated that GLP-1(7-36) infusion acutely increased muscle microvascular blood volume in the absence of changes in microvascular blood flow velocity or femoral blood flow, in association with increased plasma NO, muscle insulin clearance/uptake, hindlimb glucose extraction and muscle interstitial oxygen saturation (Chai et al., 2012). It should be noted that in contrast to the *ex vivo* studies, GLP-1(9-36) failed to modulate vascular function in rats *in vivo* when given as either a bolus dose or via short-term infusion, which together with the fact that DPP-4 inhibitors prolonged the vascular actions of native GLP-1(7-36) in this setting (Gardiner et al., 2010), suggest that the actions of this "inactive" metabolite may not be significant *in vivo*.

Importantly, it appears that the vascular effects of GLP-1 are also evident in the setting of diabetes, where they are reported to promote beneficial actions. Chronic treatment of STZ/nicotinamide T1DM rats with either GLP-1(7-36) or exendin-4 was shown to prevent endothelial dysfunction in parallel with reduction of blood glucose (Özyazgan et al., 2005), effects which may be mediated via activation of endothelial NO synthase (eNOS) (Goyal et al., 2010). Although similar studies have not been performed in the setting of overt T2DM, it was recently reported that chronic treatment of insulin-resistant Zucker rats with the DPP-4 inhibitor, linagliptin, resulted in reduced hypertension in parallel with increased expression of total/phosphorylated eNOS (Aroor et al., 2013). Furthermore, high-fat fed apolipoprotein E-deficient mice treated with a different DPP-4 inhibitor, des-fluoro-sitagliptin, demonstrated attenuation of endothelial dysfunction in parallel with eNOS activation and improved glucose tolerance (Matsubara et al., 2012). Interestingly, however, endothelial dysfunction in rat femoral artery induced by short-term triglyceride exposure was not affected by exendin-4 (Nathanson et al., 2009), suggesting that the reported *in vivo* protective actions may occur via indirect mechanisms. In this regard, it is important to note that the vascular actions of GLP-1 in diabetes are likely to occur, at least partly, secondary to stimulation of insulin, which induces vascular relaxation via Ca^{2+}-dependent activation of eNOS (Han et al., 1995; Kahn et al., 1998).

In addition to data supporting important vascular actions of GLP-1 in experimental diabetes, several studies have reported beneficial functional effects in the clinical setting. For example, in T2DM patients with stable CAD, acute GLP-1 administration improved brachial artery flow-mediated vasodilation, an effect not observed in healthy individuals (Nyström et al., 2004). Comparable effects were observed in insulin-resistant patients with obesity-related metabolic syndrome, where acute treatment with GLP-1(7-36) enhanced insulin-mediated forearm blood flow responses to both acetylcholine and sodium nitroprusside in the absence

of changes in forearm glucose extraction/uptake, whilst GLP-1(9-36) did not affect vascular function (Tesauro et al., 2013). Similarly, in patients with T1DM, brachial artery endothelial dysfunction induced by acute blood glucose modulation was counteracted by simultaneous infusion of GLP-1 (Ceriello et al., 2013). Interestingly, GLP-1-induced enhancement of endothelium-dependent peripheral vasodilatation observed in non-diabetic individuals is differentially modulated by sulphonylureas, with glyburide abolishing GLP-1 induced acetycholine-mediated responses which are unaltered by glimepiride (Basu et al., 2007). Furthermore exenatide, which is commonly used for hyperglycaemic control in T2DM, also increased postprandial endothelial function assessed by peripheral arterial tonometry in patients with recent-onset disease when given as a single dose, largely secondary to reduction of circulating triglycerides (Koska et al., 2010), although chronic treatment for 3 months in obese pre-diabetic patients had no additional effect when compared to those receiving metformin (Kelly et al., 2012). Whilst it appears that clinical GLP-1 administration exerts acute vascular effects in T2DM, data on its chronic actions in this setting are variable. T2DM patients who received exenatide for a period of 4 months as an adjunct to standard metformin therapy demonstrated improved brachial artery flow-mediated dilatation, indicated by elevated peak dilatation and shear rate which are reflective of improved macrovascular and microvascular function, respectively (Irace et al., 2013). Notably, enhanced vasodilatation in exenatide-treated patients in this study was significantly greater than that observed in those receiving glimepiride as an add-on to metformin therapy. Furthermore, liraglutide treatment for 12 weeks in T2DM patients well controlled on metformin monotherapy resulted in improvement of both circulating markers of vascular function (asymmetric dimethylarginine, ADMA; plasminogen activator inhibitor-1, PAI; E-selectin) and retinal microvascular endothelial function (Forst et al., 2012). However, a similar study in a small number of severely-obese T2DM patients chronically-treated with GLP-1R agonists for 6 months

reported that neither exenatide or liraglutide had any effect on brachial artery endothelial-dependent flow-mediated dilation (Hopkins et al., 2013), indicating potential for other confounding factors in this setting which may need to be considered. Furthermore, 6-week treatment with sitagliptin or alogliptin significantly reduced flow-mediated dilatation in male T2DM patients (Ayaori et al., 2013), suggesting that chronically-increased physiological levels of GLP-1 may exert unfavourable vascular actions, although it is possible that this could be a class-specific effect of DPP-4 inhibitors warranting further investigation.

Inflammation and atherosclerosis

It is well known that the incidence and progression of endothelial dysfunction is exacerbated in T2DM, secondary to established risk factors, such as insulin resistance, dyslipidaemia and hyperglycaemia. Endothelial dysfunction in this setting is characterised by elevation of circulating adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) vascular cell adhesion molecule-1 (VCAM-1), and an increased propensity to develop atherosclerosis which is typified by inflammatory cell infiltration and plaque formation (van Gaal et al., 2006). Interestingly, several recent clinical and experimental studies appear to indicate that GLP-1 exerts both anti-inflammatory and anti-atherogenic actions. For example, GLP-1 treatment in T2DM patients is associated with beneficial effects on a number of established CVD biomarkers, including high sensitivity C-reactive protein (hs-CRP) and plasminogen activator inhibitor-1 (PAI-1), which are important in atherosclerosis development (Haffner, 2006). Similarly, 14-week treatment of T2DM patients with liraglutide resulted in significantly reduced PAI-1 levels, and a dose-dependent decrease in plasma hs-CRP levels (Vilsbøll et al., 2007; Courrèges et al., 2008), an effect that was also observed after 26-week treatment with exenatide (Bergenstal et al., 2010), and was over and above that seen in patients treated with insulin glargine (Diamant et al., 2010). Importantly,

the beneficial effects of exenatide on circulating hs-CRP appear to persist at 1 year treatment in T2DM patients receiving standard metformin therapy, resulting in reduced levels of both hs-CRP and leptin (Bunck et al., 2010). DPP-4 inhibitors seem to exert similar effects as T2DM patients receiving sitagliptin for 6 months also demonstrated significant reductions in plasma hs-CRP, together with VCAM-1 and associated albuminuria, which may attenuate glucose excursion and inhibit vascular inflammation (Horváth et al., 2009; Hattori, 2010). Interestingly, a recent study demonstrated that cessation of exenatide treatment resulted in reversal of benefits on circulating hs-CRP within 6 months (Varanasi et al., 2011). It should be noted that although these studies support the suggestion that GLP-1 may protect against inflammation and atherosclerosis in the clinical setting, it is not possible to draw clear conclusions due to the absence of longer-term studies specifically assessing effects on disease development. Interestingly, a recent study has reported a positive correlation between circulating GLP-1 levels and CAD in both diabetic and non-diabetic patients undergoing angiography due to typical or atypical chest pain, highlighting the possibility that GLP-1 may exert detrimental effects in this setting (Piotrowski et al., 2013).

Nonetheless, the majority of clinical data are broadly supportive of anti-inflammatory actions of GLP-1, which persist for up to 12 weeks in obese T2DM patients after a single exenatide injection (Chaudhuri et al., 2011). Indeed, in this study GLP-1 was associated with specific reduction of several inflammatory mediators, including tumour necrosis factor-α (TNF-α), toll-like receptor-2 (TLR-2) and TLR-4, in parallel with suppression of nuclear factor kappa-light-chain-enhancer of activated B (NF-κβ) signalling and matrix metalloproteinase-9 (MMP-9) activity, which are key initiating factors of atherosclerosis. Furthermore, in obese T2DM patients, 8-week liraglutide treatment is reported to decrease levels of the inflammatory macrophage activation molecule, sCD163, and pro-inflammatory cytokines, TNF-α and interleukin-6, whilst increasing levels of the anti-inflammatory

adipokine, adiponectin (Hogan et al., 2014). Importantly, these clinical observations are supported by a number of experimental studies which have specifically assessed the effects of GLP-1 on development and progression of atherosclerosis. For example, continuous infusion of exendin-4 in both wild-type and apolipoprotein E-deficient normoglycaemic mice was reported to decrease monocyte adhesion and development of atherosclerotic lesions in thoracic aorta, effects proposed to occur via cAMP/PKA-dependent suppression of inflammation (Arakawa et al., 2010). These findings were confirmed by a different group who demonstrated reduced aortic macrophage recruitment, foam cell formation and atherosclerotic lesion development in apolipoprotein E-deficient mice after GLP-1 infusion (Nagashima et al., 2011). Furthermore, chemokine-induced migration of $CD4^+$ lymphocytes is inhibited by both GLP-1(7-36) and exendin-4 in a GLP-1R dependent manner (Marx et al., 2010), whilst liraglutide supresses NF-κβ signalling in human umbilical vein endothelial cells (HUVECs) and THP-1 monocyte adhesion in human aortic endothelial cells via downstream activation of several pro-inflammatory and cell adhesion molecules, including TNF-α, VCAM-1 and E-selectin (Shiraki et al., 2012; Krasner et al., 2014). Indeed, liraglutide inhibits TNFα in human vascular endothelial cells and reduces hyperglycaemia-mediated PAI-1, ICAM-1 and VCAM-1 activation, which is associated with endothelial dysfunction and accelerated atherogenesis in T2DM (Liu et al., 2009). Furthermore, the DPP-4 inhibitor, des-fluoro-sitagliptin, is reported to exert cAMP-dependent anti-inflammatory actions in cultured human macrophages by increasing GLP-1 levels and to reduce atherosclerotic lesion formation in apolipoprotein E-deficient mice (Matsubara et al., 2012), whilst alogliptin inhibits vascular monocyte/macrophage recruitment and reduces atherosclerotic burden in high-fat fed LDL receptor-deficient mice in association with improvement of metabolic indices (Shah et al., 2011). Taken together, these experimental data clearly support an important role for GLP-1 in protecting against vascular inflammation and atherogenesis, effects which are borne out by

the reported clinical benefits of GLP-1 treatment on circulating inflammatory mediators and CVD biomarkers. However, it is evident that long-term studies specifically investigating effects on atherosclerotic disease development and progression are required to ascertain whether the apparent protective effects of GLP-1 under both normoglycaemic and diabetic conditions may translate to the clinical setting.

Angiogenesis

Abnormal angiogenesis is a hallmark of CVD which is exacerbated by diabetes, with impaired neovascularisation contributing significantly to progression of ischaemic disease associated with peripheral and coronary arteries. Interestingly, it is becoming apparent that GLP-1 may modulate angiogenesis suggesting that such actions may underlie some of its reported beneficial cardiovascular effects. For example, exendin-4 stimulates proliferation of human coronary artery endothelial cells in a GLP-1R dependent manner via downstream activation of eNOS, PKA and PI3K/Akt signalling (Erdogdu et al., 2010) and promotes *in vitro* HUVEC migration, *ex vivo* aortic sprouting angiogenesis, and *in vivo* blood vessel formation in Matrigel plugs (Kang et al., 2013), whilst native GLP-1(7-36) stimulates *in vitro* angiogenesis in HUVECs via Akt, Src and protein kinase C-dependent pathways (Aronis et al., 2013), suggesting that GLP-1 may directly modulate neovascularisation. Importantly, these effects appear to translate to the pathological situation, with several recent studies reporting that GLP-1 can promote the pro-angiogenic actions of mesenchymal stem cells in different disease settings. Intra-coronary artery delivery of GLP-1 eluting encapsulated human mesenchymal stem cells in a porcine model of experimental myocardial infarction (MI) resulted in improved left ventricular function and remodelling which was associated with increased infarct zone angiogenesis (Wright et al., 2012), whilst peri-adventitial treatment of porcine vein grafts with these cells inhibited neointima formation in parallel with accelerated

adventitial angiogenesis (Huang et al., 2013). Furthermore, the addition of GLP-1 to encapsulated human mesenchymal cells significantly improved blood flow recovery and foot salvage in a mouse model of hindlimb ischaemia via increased capillary and arteriole formation secondary to paracrine activation of vascular endothelial growth factor-A (Katare et al., 2013). Although the majority of work investigating the pro-angiogenic actions of GLP-1 has been performed in normoglycaemic models, a recent study reported similar beneficial effects in the setting of diabetes. Impaired myocardial angiogenesis in STZ-treated T1DM rats and associated fibrosis and diastolic dysfunction were reversed by genetic deletion of DPP-4 or pharmacological inhibition with vildagliptin (Shigeta et al., 2012). Interestingly, this study identified DPP-4 as being membrane-bound and localised to the cardiac capillary endothelium with increased expression in diabetes, which together with a report of increased binding affinity of GLP-1 to the coronary endothelium but not cardiomyocytes in isolated perfused T1DM rat hearts (Barakat et al., 2011), supports a key endothelial-specific role of GLP-1 in this setting. Although these data provide supportive evidence for pro-angiogenic actions of GLP-1 in diabetes, it is clear that additional mechanistic studies are required using different CVD models in order to define its precise role. Furthermore, it is important to assess the effects of GLP-1 therapy in diabetic patients in order to investigate whether the apparent pro-angiogenic effects of GLP-1 translate to the clinical setting and are of functional relevance.

GLP-1 AND THE DIABETIC MYOCARDIUM

The heart is one of the major organ targets of GLP-1 and an increasing number of studies have investigated the actions of native GLP-1(7-36), GLP-1R agonists and DPP-4 inhibitors in the context of cardioprotection. The majority of experimental studies have focused on the effects of GLP-1 in cardiac ischemia and its apparent ability to protect against acute myocardial damage. Indeed, it is well established that GLP-1 pre-treatment and chronic

DPP-4 inhibition reduce infarct size after experimental ischaemia in both small and large animal models, which is associated with increased survival and improved cardiac function (Bose et al., 2005; Ban et al., 2008, 2010; Timmers et al., 2009). Interestingly, a recent study employing a rabbit model of ischaemia/reperfusion injury, reported protective actions of transferrin-stabilised GLP-1, when given both 12 hours prior to ischaemia and immediately upon reperfusion, suggesting that GLP-1 may limit infarct size and contractile dysfunction directly, rather than by pre-conditioning the heart against ischaemia, as suggested by previous reports (Matsubara et al., 2011). In addition to its established beneficial actions against acute ischaemic myocardial damage, GLP-1 also confers protection against contractile dysfunction associated with experimental chronic post-MI remodelling, dilated cardiomyopathy and hypertensive heart failure (Nikolaidis et al., 2004a; Poornima et al., 2008; Liu et al., 2010), with similar results reported in the clinical setting in response to short-term GLP-1 treatment (Nikolaidis et al., 2004b; Sokos et al., 2006).

Until recently, only limited data were available on the cardiac actions of GLP-1 in diabetes, but it is becoming increasingly apparent that GLP-1 also plays a key cardioprotective role in this setting. This is important as it is well known that hyperglycaemia is associated with increased susceptibility to cardiac disease and poor outcomes in both humans and experimental models (Shiomi et al., 2003; Liu et al., 2005; Greer et al., 2006; Vergès et al., 2007). Chronic DPP-4 inhibition with linagliptin improves obesity-related diastolic dysfunction in insulin-resistant Zucker rats, but has no effect on cardiomyocyte hypertrophy and fibrosis (Aroor et al., 2013). Indeed, exendin-4 has been reported to directly protect isolated rat cardiomyocytes from high glucose-induced apoptosis via inhibition of endoplasmic reticulum stress and activation of sarcoplasmic reticulum Ca^{2+} ATPase 2a (Younce et al., 2013). GLP-1 also appears to protect against diabetic cardiomyopathy, which is defined as cardiac dysfunction in the absence of, or disproportionate to, associated

hypertension and CAD, and is characterised by marked collagen accumulation and impaired diastolic function (Bugger and Abel, 2014). Both GLP-1R activation and DPP-4 inhibition attenuate development of cardiac dysfunction, extracellular matrix remodelling, cardiomyocyte hypertrophy and apoptosis in experimental models of T1DM and T2DM, with various mechanisms proposed including reduction of lipid accumulation, oxidative stress and myocardial inflammation, and modulation of the MMP-2/tissue inhibitor of MMP-2 axis, endoplasmic reticulum stress and microvascular barrier function (Shigeta et al., 2012; Liu et al., 2013; Monji et al., 2013; Picatoste et al., 2013; Wang et al., 2013). Furthermore, it appears that GLP-1 also confers infarct-reducing actions in diabetes, which is associated with increased susceptibility to myocardial ischaemia. For example, mice made diabetic by a combination of STZ injection and high-fat feeding and treated with the GLP-1R agonist, liraglutide, prior to coronary artery ligation, demonstrated reduced infarct development and improved survival compared to those treated with the glucose-lowering drug, metformin, suggesting that the observed effects occurred via direct actions on the heart and not secondary to reduced blood glucose (Noyan-Ashraf et al., 2009). Similar cardioprotective effects have been reported with DPP-4 inhibition in experimental diet-induced obesity (Huisamen et al., 2013), whilst the infarct-limiting effects of exendin-4 in mice with T2DM were shown to be mediated by cAMP-induced PKA activation (Ye et al., 2013). Interestingly, it has recently been suggested that the infarct-reducing actions of DPP-4 inhibitors may be glucose-dependent, as both sitagliptin and vildagliptin were found to only decrease infarct size in isolated rat hearts subjected to ischaemia-reperfusion injury when they were perfused with elevated glucose concentrations ≥7mmol/L, with similar results observed *in vivo* in diabetic, but not normoglycaemic rats (Hausenloy et al., 2013). This raises the intriguing possibility that glucose-lowering may counteract the cardioprotective actions of GLP-1 and explain why several large-scale clinical trials focused on intensive glucose control in T2DM have failed to

demonstrate significant cardiovascular benefits (Giorgino et al., 2013). Furthermore, it appears that at least part of the observed beneficial actions of DPP-4 inhibitors against ischaemia-reperfusion injury may be mediated by the chemokine, stromal cell-derived factor 1α, in a GLP-1 independent manner (Bromage et al., 2014).

In addition to the experimental data highlighting a protective role for GLP-1 in the diabetic heart, importantly, a small number of studies have assessed its cardiac actions in patients with diabetes. It has been known for some time that short-term GLP-1 treatment exerts beneficial effects in clinical heart failure in both normoglycaemic and diabetic patients. For example, in a small number of heart failure patients (New York Heart Association class III/IV), 5-week infusion with GLP-1 plus standard therapy improved left ventricular ejection fraction and myocardial oxygen consumption compared to those receiving standard therapy alone, effects that were seen in both diabetic and non-diabetic patients (Sokos et al., 2006). Furthermore, a small non-randomised trial of 72-hour GLP-1 infusion following primary angioplasty after acute MI led to improved cardiac function in both non-diabetic and diabetic patients which was still evident upon 120 day follow-up (Nikolaidis et al., 2004b). More recently, a larger randomised trial in patients presenting with ST-segment elevation MI reported that exenatide infusion for 15 minutes prior to primary angioplasty continued until 6 hours post reperfusion resulted in improved myocardial salvage at 3 months although no functional benefits were observed (Lønborg et al., 2012). Indeed, two current clinical trials are assessing the potential of using exenatide as a post-conditioning agent to reduce reperfusion injury following percutaneous coronary intervention (Effect of Additional Treatment With EXenatide in Patients With an Acute Myocardial Infarction, the EXAMI trial, NCT01254123; Pharmacological Postconditioning to Reduce Infarct Size Following Primary PCI, POSTCON II, NCT00835848). Interestingly, in patients with left ventricular diastolic dysfunction, DPP-4 activity in the coronary sinus and peripheral circulation is reported to be negatively correlated

with diastolic function and increased by comorbid diabetes (Shigeta et al., 2012), suggesting that reduced GLP-1 levels in diabetes may underlie associated cardiac dysfunction. Exenatide has also been found to modulate myocardial glucose transport and uptake in T2DM patients dependent upon the degree of insulin resistance (Gejl et al., 2012), although a similar study reported that GLP-1 induced increases in resting myocardial glucose uptake in lean individuals were absent in obese T2DM patients, with parallel studies in pigs suggesting that this was due to impaired p38-mitogen-activated protein kinase (MAPK) signalling (Moberly et al., 2013). Interestingly, a recent experimental study found that exendin-4 reduced contractile function and was unable to stimulate glucose utilisation in normal rat hearts in the presence of fatty acids (Nguyen et al., 2013), despite previous reports of increased myocardial glucose uptake in response to GLP-1 in experimental myocardial ischaemia and dilated cardiomyopathy (Nikolaidis et al., 2005; Zhao et al., 2006; Bhashyam et al., 2010). Such findings highlight the need for detailed investigation of the effects of GLP-1 on altered myocardial metabolism in diabetic patients both with and without cardiac complications, in which the effects of GLP-1 may diverge.

Although these clinical and experimental data are clearly supportive of an important role for GLP-1 signalling in the diabetic heart, they are largely descriptive with limited focus on underlying mechanisms. Previous studies in experimental models of heart failure have highlighted several pathways which may mediate the cardioprotective effects of GLP-1, including cAMP/PKA, PI3K/Akt, p44/p42MAPK, ERK1/2 (Bose et al., 2005; Timmers et al., 2009; Ravassa et al., 2011), together with suggestions of GLP-1R independent signalling (Nikolaidis et al., 2005; Sonne et al., 2008). However, the precise mechanisms underlying the apparent protective actions of GLP-1 in the diabetic heart, in which GLP-1 signalling is likely to be different, remain unknown and clearly need to be defined in order to fully assess the therapeutic potential of GLP-1 in this setting.

SUMMARY AND FUTURE PERSPECTIVE

Over recent years it has become clear that GLP-1 exerts important actions on the cardiovascular system in both health and disease in addition to its prototypic effects on glycaemic control (Grieve et al., 2009) and also confers beneficial effects on the cardiovascular risk profile in diabetic patients. However, emerging evidence now strongly suggests that GLP-1 exerts specific cardiovascular actions in diabetes and may attenuate the development and progression of associated cardiovascular complications (summarised in Figure 1), although the precise mechanisms are yet to be established with several candidate pathways proposed (see Figure 2). Nonetheless, it is important to note that the majority of data pointing towards such beneficial effects are largely experimental and that equivalent information on the cardiovascular actions of GLP-1 in the clinical setting of diabetes is somewhat lacking, particularly in relation to its chronic effects. In this regard, the first large-scale GLP-1 clinical trials to assess cardiovascular outcomes after long-term treatment (SAVOR-TIMI 53, EXAMINE) have recently reported that chronic DPP-4 inhibition with either saxagliptin or apogliptin had no significant effects on their primary composite end points of cardiovascular death, or non-fatal MI/stroke (Scirica et al., 2013; White et al., 2013), although it should be noted that SAVOR-TIMI 53 reported a 27% increase in hospitalisation for heart failure. However, until the results of several ongoing clinical trials investigating the chronic cardiovascular effects of GLP-1R agonists are known (summarised in Table 1 together with further DPP-4 inhibitor trials), in which circulating GLP-1 levels will be much higher than those achieved using DPP-4 inhibitors, no conclusions can be drawn on either the potential clinical cardiovascular benefits or safety of GLP-1 based therapies in diabetes. Nonetheless, the apparent complexity of cardiovascular GLP-1 signalling under both normal and diabetic conditions clearly suggests that it is likely that selective targeting of specific

aspects of CVD may be required in order to realise optimal benefits of GLP-1 targeting in this setting.

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STATEMENT OF CONFLICTS OF INTEREST

None.

AUTHORSHIP CONTRIBUTION

M.T. drafted the manuscript; A.C. wrote the vascular function section; E.R. and B.D.G. planned and critically reviewed the manuscript; D.J.G. prepared the final manuscript.

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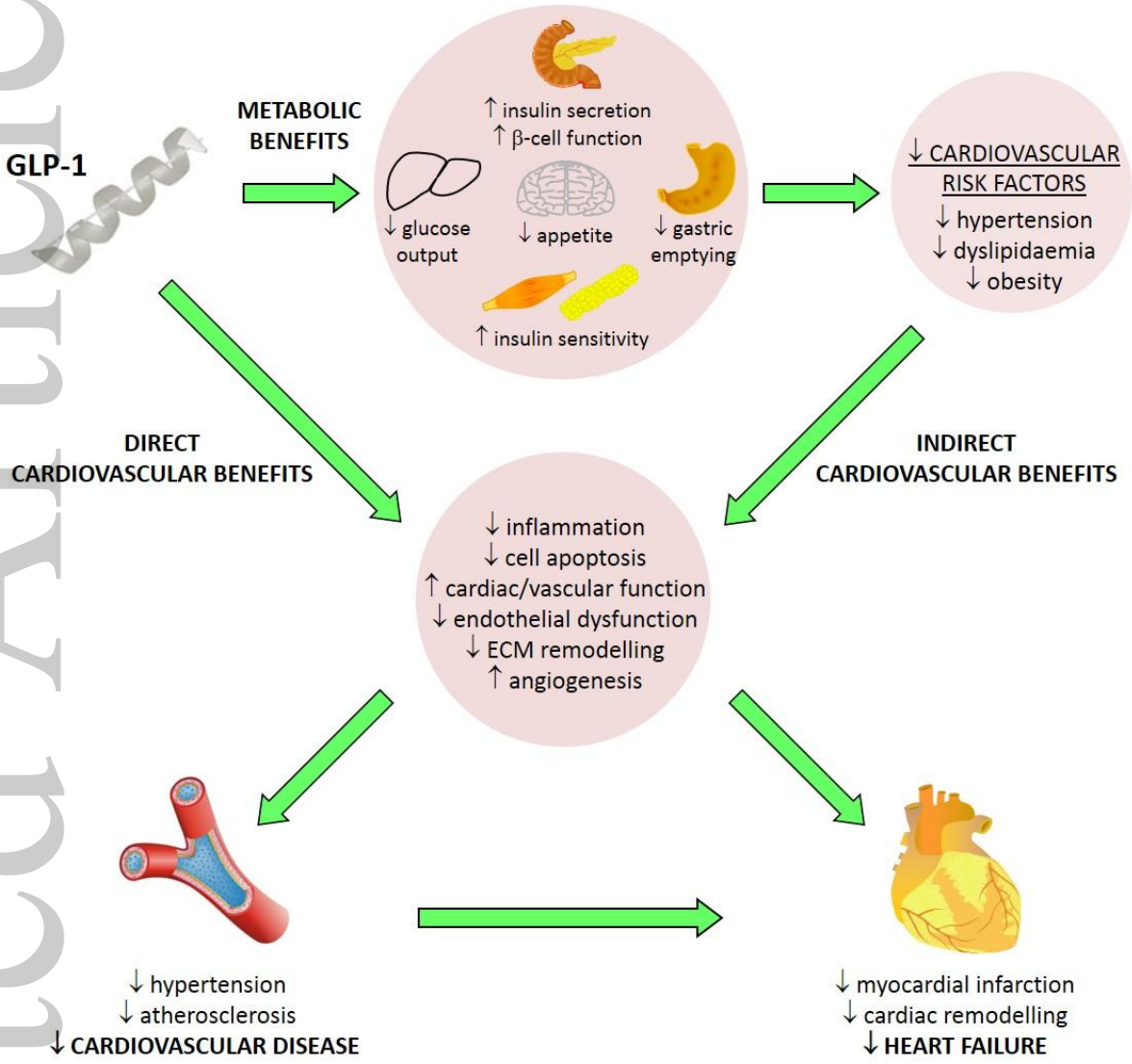
FIGURE LEGENDS

Figure 1 Summary of the cardiovascular actions of GLP-1 in diabetes. GLP-1 exerts indirect cardiovascular benefits in diabetes secondary to its established metabolic actions and subsequent reduction of cardiovascular risk factors. In addition, GLP-1 promotes direct cardiovascular benefits which confer protection against CVD and heart failure, the latter of which may occur via direct myocardial actions or secondary to reduced hypertension and coronary atherosclerosis.

Figure 2 Proposed mechanisms underlying the reported cardiovascular actions of GLP-1. Although it is well established that GLP-1 exerts several beneficial effects on the cardiovascular system relevant to diabetes, such as reduction of metabolic CVD risk factors, blood pressure modulation, improved vascular function, decreased atherosclerosis, promotion of angiogenesis, and attenuation of adverse cardiac remodelling, the precise mechanisms are yet to be established, although several pathways have been proposed which are the focus of further investigation. EC, endothelial cell; ECM, extracellular matrix; PKC, protein kinase C; SDF, stromal cell-derived factor 1α; SR, sarcoplasmic reticulum; VEGF, vascular endothelial growth factor.

TABLE 1: Current long-term clinical trials of GLP-1 based therapies on cardiovascular outcomes

Study name	Drug	Administration	Estimated patient enrolment	Primary outcome(s)	Expected end date	NCT identifier
EXSCEL	Exenatide	Subcutaneous injection, 2 mg, once weekly	14,000	Rate of CV death, non-fatal MI or non-fatal stroke	December 2017	NCT01144338
LEADER	Liraglutide	Subcutaneous injection, 1.8 mg, once daily	9,340	Time to CV death, non-fatal MI or non-fatal stroke	October 2015	NCT01179048
ELIXA	Lixisenatide	Subcutaneous injection, 20 μg, once daily	6,000	Time to first primary CV event	January 2015	NCT01147250
REWIND	Dulaglutide	Subcutaneous injection, 1.5 mg, once weekly	9622	Time to CV death, non-fatal MI, or non-fatal stroke	August 2019	NCT01394952
TECOS	Sitagliptin	Oral, 50 or 100 mg, once daily	14,000	Time to first CV event	December 2014	NCT00790205
CAROLINA	Linagliptin	Not specified	8,300	Time to CV death, non-fatal MI, non-fatal stroke and hospitalisation for unstable angina pectoris	January 2018	NCT01897532
CARMELINA	Linagliptin	Oral, 5 mg, once daily	6,000	Time to CV death, non-fatal MI, non-fatal stroke and hospitalisation for unstable angina pectoris	September 2018	NCT01243424



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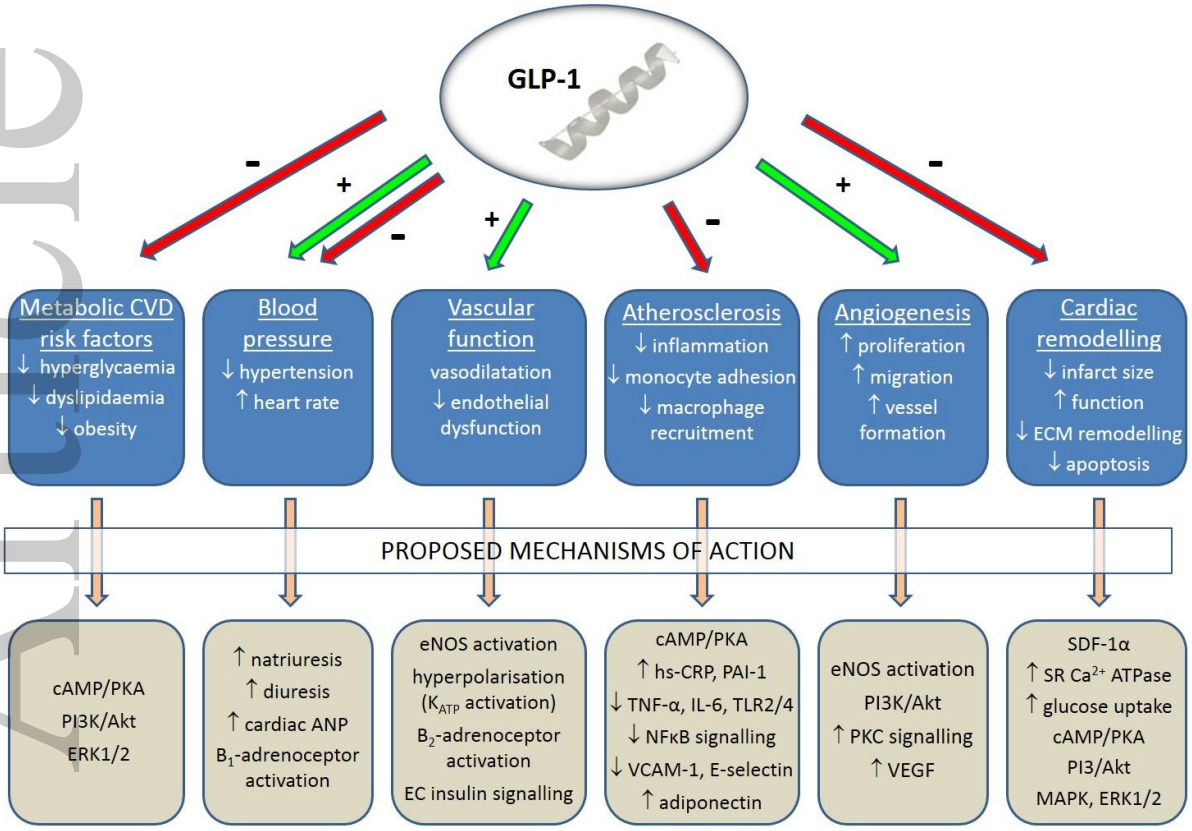

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